Injury-induced endotheliopathy: What you need to know

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ABSTRACT: The endotheliopathy of trauma involves a complex interplay between the glycocalyx, von Willebrand factor, and platelets that leads to abnormalities in coagulation, inflammation, and endothelial cell (EC) function. The current review presents a synopsis of EC function under homeostatic conditions, the structure and function of the endothelial glycocalyx; mechanisms of EC injury and activation after trauma; pathological consequences of the EoT at the cellular level; and clinical implications of the EoT. Recent evidence is presented that links the EoT to extracellular vesicles and hyperadhesive ultralarge von Willebrand factor multimers through their roles in coagulopathy. Lastly, potential therapeutics to mitigate the EoT are discussed. Most research to date has focused on blood products, primarily plasma, and its contribution to restoring postinjury EC dysfunction. Additional therapeutic adjuvants that target the glycocalyx, ultralarge von Willebrand factor, low ADAMTS-13, and pathologic extracellular vesicles are reviewed. Much of the pathobiology of EoT is known, but a better mechanistic understanding can help guide therapeutics to further repair the EoT and improve patient outcomes. (*J Trauma Acute Care Surg.* 2023;95: 454–463. Copyright © 2023 Wolters Kluwer Health, Inc. All rights reserved.)

KEY WORDS: Endotheliopathy of trauma; vonWillebrands factor; extracellular vesicles; glycocalyx; syndecan.

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OVERVIEW OF THE ENDOTHELIUM

Endothelial Regulation of Coagulation, Inflammation, and Vascular Barrier Function

Under physiologic conditions, endothelial cells (ECs) actively suppress coagulation by providing an antithrombotic surface to maintain blood fluidity. The most important endothelial-dependent anticoagulant mechanisms include expression of (1) heparan sulfate proteoglycans, (2) thrombomodulin (TM), (3) endothelial protein C receptor (EPCR), and (4) tissue factor pathway inhibitor (Fig. 1). Heparan sulfate is a glycosaminoglycan (GAG) bound to EC surface proteoglycans, including syndecan and glypican. Its anticoagulant function is mediated through interaction with circulating antithrombin (AT) via binding to a unique pentasaccharide domain, thereby localizing AT to the vessel wall and accelerating AT-mediated inhibition of both activated factor Xa and thrombin (IIa). Thrombomodulin is an EC surface glycoprotein that, upon high affinity binding to thrombin, will change the substrate specificity of thrombin from procoagulant cleavage of fibrinogen and FV, to anticoagulant cleavage of protein C to generate activated protein C. This reaction is accelerated when protein C is bound to EPCR, bringing protein C into close proximity to the TM-thrombin complex. Tissue factor pathway inhibitor potently inhibits the initiation of extrinsic coagulation through its inactivation of the tissue factor (TF)–factor VIIa complex.[1] Endothelial cells also express antiplatelet molecules, such as nitric oxide and prostacyclin, which suppress platelet activation.

The endothelium contributes to primary hemostatic clot formation through expression of von Willebrand factor (VWF) on its surface. Upon exposure to subendothelial proteins such as collagen, VWF tethers platelets to the injured vasculature through binding of its A3 domain to the subendothelial collagen and its A1 domain to the GP Ib-IX-V complex on platelets. Endothelial cells propagate thrombus formation through their role in secondary hemostasis, which is mediated in part through expression of TF, by interacting with cofactor factor VIIa to activate FX to generate thrombin. To regulate thrombus growth and vessel patency, ECs also critically regulate fibrinolysis through their expression of tissue and urokinase plasminogen activators (tPA and uPA, respectively). Both tPA and uPA cleave plasminogen to produce plasmin, which degrades fibrin cross-linkages. These enzymes are inhibited by EC-derived plasminogen activator inhibitor type I, thereby inhibiting fibrinolysis.

Suppressing coagulation is an indirect mechanism, through which ECs regulate inflammation, because of cross-talk between coagulation and immune mechanisms.[2] Similar to their role in platelet inhibition, ECs also directly control activation of proinflammatory processes through secretion of nitric oxide and prostacyclin, which reduce leukocyte recruitment, adhesion, and activation to the vessel wall. Production of nitric oxide also maintains EC quiescence through inhibition of proinflammatory gene transcription. Endothelial cells also prevent leukocyte adhesion through sequestration of P-selectin within its Weibel-Palade bodies. However, in response to an injury or insult, ECs undergo rapid Type I activation, characterized by exteriorization of leukocyte-interactive chemokines and degranulation of the Weibel-Palade bodies.[3] This is followed by a more sustained Type II response, which includes de novo transcription of cytokines (interleukins and monocyte chemoattractant protein) and chemokines including E-selectin, intracellular adhesion molecule 1, and vascular cell adhesion molecule 1.[4]

Finally, ECs play an essential role in maintaining the semipermeable barrier between blood and tissue. The endothelium uses two distinct pathways for regulating molecular transport: (1) the transcellular pathway and (2) the paracellular pathway. The transcellular pathway regulates passage of large molecules, such as albumin, through caveolae, which are lipid rafts that transport macromolecules into and through the endothelial cytoplasm. The paracellular pathway, on the other hand, regulates passage of small molecules (i.e., water, ions). The paracellular pathway is composed of interendothelial junctions, including gap, adherens, and tight junctions. Connexins are gap junctional proteins that mediate the direct transfer of calcium ions, current, and signaling molecules between neighboring cells. Adherens junctions are formed by paracellular adhesion of vascular endothelial cadherin, which regulates molecular transport and barrier function through interaction with the actin cytoskeleton via binding catenins. Tight junctional proteins include occludin, claudin, and junctional adhesion molecules, which regulate permeability through zona occludens–mediated linkages with

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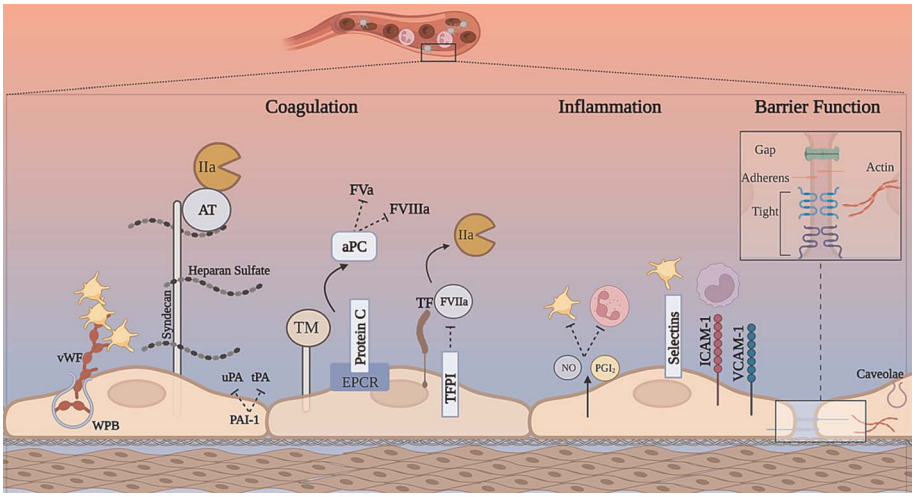

Figure 1. Endothelial mechanisms that regulate coagulation, inflammation, and vascular barrier integrity. Endothelial cells control activation of the coagulation system by balancing both procoagulant and anticoagulant and fibrinolytic processes. Key anticoagulant mechanisms include (1) expression of heparan sulfates, which are bound to a syndecan core and bind AT to inhibit thrombin (IIa); (2) expression of TM and EPCR, which cooperatively generate aPC; and (3) expression of TFPI, which suppresses thrombin generation through inhibition of the TF-FVIIa complex. Endothelial cells can potently activate the coagulation system through secretion of VWF, which tethers platelets to the subendothelium and expresses TF. Clot dissolution is regulated through expression of profibrinolytic enzymes tissue and urokinase plasminogen activators (tPA and uPA, respectively) and antifibrinolytic PAI-1. Endothelial cells prevent adhesion and activation of circulating platelets and immune cells through secretion of nitric oxide and PGI_2. They can also selectively mediate cellular adhesion through expression of selectins and intracellular or vascular adhesion molecules (ICAM-1 and VCAM-1, respectively). Endothelial barrier function is regulated by the transcellular pathway through calveolae and the paracellular pathway through expression of gap, adherens, and tight junctional proteins (image created with BioRender.com). aPC, activated protein C; FVIIa, factor VIIa; ICAM-1, intracellular adhesion molecule 1; PAI-1, plasminogen activator inhibitor type 1; PGI_2, prostacyclin; TFPI, tissue factor pathway inhibitor; VCAM-1, vascular cell adhesion molecule 1.

the actin cytoskeleton. Inflammatory mediators result in direct dissociation/reorganization of adherens junction, reduction in EC expression of interendothelial junctional proteins, or induce actin stress fiber formation, which can all result in disruption of a healthy endothelial barrier, with increases in paracellular permeability, and, ultimately, tissue edema.

Structure and Function of the Endothelial Glycocalyx

Positioned at the interface between blood and the endothelial surface, the glycocalyx is central to maintenance of a healthy endothelium (Fig. 2*A*). The mesh-like glycocalyx layer is composed of interwoven proteoglycans and glycoproteins to which are bound an array of GAG chains. The syndecan and glypican proteoglycans express heparan sulfate, the most abundant GAG, as well as hyaluronan, chondroitin sulfate, and dermatan sulfate. The transmembrane glycoprotein, CD44, serves as the receptor for hyaluronan. Collectively, the glycocalyx maintains vascular homeostasis and preserves normal EC function by serving as a physical impediment to circulating platelets, leukocytes, and soluble EC activating factors to the underlying endothelium.[5] Heparan sulfate and chondroitin sulfate contribute to the anticoagulant tone of the endothelium through interaction and activation of AT and cooperative interactions with TM that augment its affinity for binding thrombin, respectively. The lesser abundant GAG, dermatan sulfate, can accelerate the thrombin inhibitory activity of heparin cofactor II. Hyaluronan modulates the chemokine gradient at the EC surface and regulates permeability through its interaction with angiopoietin 1.[6] In addition to their GAG-dependent roles, syndecans also play a key role in maintaining the endothelial barrier through their core structural position and mediating outside-in signaling events.[7]

THE ENDOTHELIOPATHY OF TRAUMA

The vascular endothelium plays a central role in maintaining organ homeostasis through its regulation of vascular tone, coagulation, inflammation, and barrier function. As such, dysregulation of normal endothelial function is an important pathological feature of organ dysfunction during disease states. The term *endotheliopathy of trauma* (EoT) is used to describe EC injury, activation, and maladaptive responses to major injuries including tissue trauma, hemorrhagic shock, and burns.[8] Importantly, clinical evidence of EoT is strongly linked with poor outcomes, indicating that EoT is an initiating pathological event contributing to systemic microvascular thrombosis, inflammation, loss of barrier integrity, and coagulopathy that are all key hallmarks of organ failure in this population (Fig. 2*B*).[9] Therefore, understanding the underlying mechanisms contributing to EoT and therapeutic strategies to limit or reverse its development could significantly improve survival and recovery from major trauma.

Postinjury Morbidity and Mortality Linked to EoT

Clinically, EoT has been identified using known plasma/serum biomarkers of EC damage, including soluble TM, syndecan-1, and E-selectin. Haywood-Watson et al.[10] and Johansson et al.[11] provided the first clinical data demonstrating EoT, noting pronounced elevations in plasma syndecan-1 upon hospital

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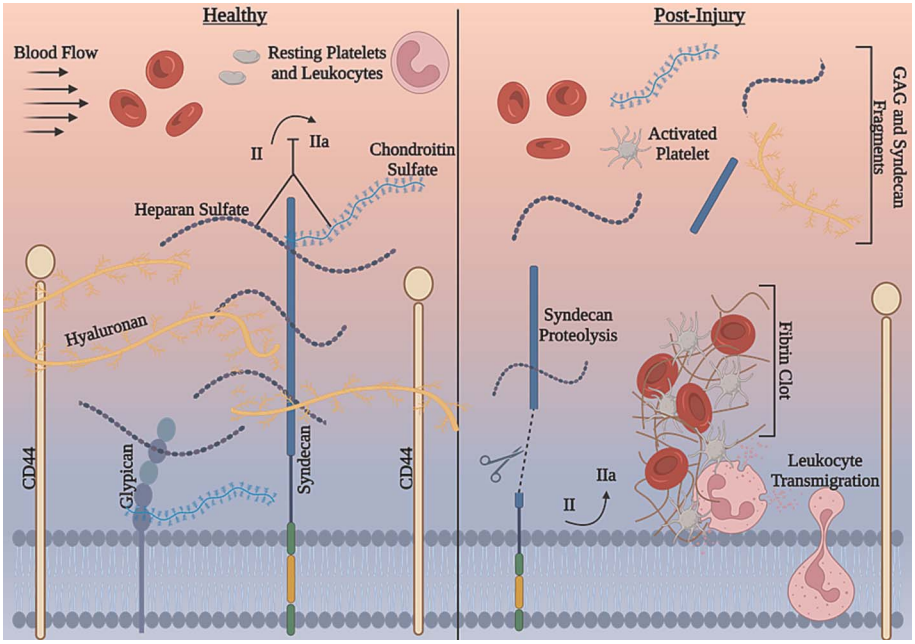

Figure 2. Endothelial glycocalyx structure and function during healthy and postinjury states. Under healthy conditions, the endothelial glycocalyx is composed of proteoglycans (syndecans) and glycoproteins (CD44 and glypican) that are interconnected by an array of GAGs, including heparan sulfate, hyaluronan, and chondroitin sulfate. When intact, the glycocalyx functionally suppresses production of thrombin (IIa) from prothrombin (II), prevents platelet and leukocyte adhesion to the endothelial surface, and regulates molecular transport and transduction of shear stress signals. However, following major injury and hemorrhagic shock, syndecan and GAGs are enzymatically cleaved from the cell surface by sheddases, resulting in release of these fragments into circulation. Loss of the endothelial glycocalyx layer contributes to coagulation at the cell surface, loss of vascular barrier integrity, and leukocyte adhesion and transmigration (image created with BioRender.com).

admission in severely injured patients. Since then, many studies have corroborated these findings and strongly linked EoT biomarkers with morbidity and mortality after major trauma. Johansson et al.[9] measured EoT in 424 level 1 trauma patients and demonstrated significant correlations between EoT and sympathoadrenal activation, as well as both early and late mortality. Gonzalez Rodriguez et al.[12] demonstrated that admission EoT, defined by a plasma syndecan-1 level ≥40 ng/mL, was associated with a 2.23 adjusted odds of in-hospital mortality compared with those without EoT. All of these studies have shown that EoT is most apparent in those patients with the highest injury severity scores and greatest metabolic indices of shock. Interestingly, recent evidence indicates that EoT occurs very rapidly after trauma with data from Naumann et al.[13] demonstrating increased soluble TM and syndecan in the prehospital setting, within minutes after injury. Additional recent data show that EoT is a pathophysiologic state that persists over the first 24 hours after injury and remains a significant predictor of poor outcomes throughout this time period.[14] Using innovative proteomics approaches, Krocker et al.[15] have shown that EoT is associated with a unique plasma proteome signature enriched in damage-associated molecular patterns (DAMPS), inflammatory mediators, and organ-specific intracellular markers, corroborating clinical evidence linking EoT with organ damage.

Despite the robust clinical relationship between EoT and trauma-associated morbidity and mortality, the cellular and plasma triggers of EoT and precise molecular events that drive EoT are still under preclinical investigation. Hereinafter, we describe the most recent data that highlight mechanisms and mediators of EoT that provide a future path forward for therapeutic development.

Glycocalyx Damage in the Development of EoT

Clinical data demonstrating pronounced shedding of EC surface components into the circulation hold key insights into mechanisms driving EoT. Loss of the endothelial glycocalyx is emerging as a unifying mechanism linking EoT with microvascular inflammation, barrier leakage, coagulopathy, and organ injury. Rahbar et al.[16] demonstrated prominent shedding of both syndecan-1 and GAG (heparan sulfate, chondroitin sulfate, and hyaluronan) components of the glycocalyx after major trauma. These findings are validated by preclinical animal data demonstrating pronounced thinning of the entire glycocalyx layer after induction of hemorrhagic shock.[17,18] It is important to note that autologous shedding of some membrane-associated glycoproteins, such as TM or EPCR, are physiologic responses to EC activation that ensure appropriate hemostasis; however, loss of EC glycocalyx components is thought to result from soluble and cell-bound sheddases. During major trauma and hemorrhagic shock, large quantities of proinflammatory mediators are released from the damaged tissue into the plasma. Cytokines such as tumor necrosis factor α instigate the release of proteases that degrade glycocalyx structures from activated leukocytes, platelets, ECs, and mast cells. This includes matrix metalloproteinases (MMPs) that nonspecifically cleave syndecans, with identified roles for MMPs 2, 9, 13, and 15 during chronic or inflammatory

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disease states.[19] In addition, activation of the coagulation system may contribute to shedding of the glycocalyx as thrombin, plasmin, and elastase all act as sheddases in vitro.[20] Given the important role of syndecans as core proteoglycans, shedding of syndecans destabilizes the glycocalyx as a whole and contributes to loss of EC surface GAG expression. In addition, the GAG-specific enzyme, heparanase I, cleaves heparan sulfates from the proteoglycan core.[21] Soluble heparan sulfate fragments can further propagate EoT by acting as damage-associated molecular patterns that signal through toll-like receptors.[22] The GAG hyaluronidase is secreted by activated leukocytes and degrades hyaluronan from the CD44 receptor, further destabilizing the glycocalyx layer. Neutrophil elastase has also been shown to cleave hyaluronan.[20] Nonenzymatic depolarization of GAGs by reactive oxygen species further contributes to nonspecific GAG shedding.[23]

Systemic shedding of the EC glycocalyx layer has a number of important pathologic consequences that contribute to EoT and subsequent organ injury. First, loss of anticoagulant GAG molecules from the cell surface creates a procoagulant environment. Syndecan proteolysis, as well as enzyme-specific cleavage of GAGs, results in loss of EC expression of heparan, chondroitin, and dermatan sulfates that collectively suppress the generation or activity of thrombin at the cell surface, thereby contributing to microvascular thrombosis. Second, shedding of the glycocalyx results in loss of a physical barrier between soluble or cellular inflammatory mediators and the endothelium. This then allows binding of soluble cytokines and DAMPs to EC receptors that induce intracellular signaling events (i.e., activation of nuclear factor κB) that increase EC prothromboinflammatory gene transcription. In addition, circulating activated leukocytes are now able to adhere to the EC surface where they release proteases and reactive oxygen species that further degrade membrane-bound proteins, as well as neutrophil extracellular traps that facilitate formation of fibrin-rich microvascular clots. Third, glycocalyx shedding results in loss of vascular barrier integrity and hyperpermeability. This results, in part, owing to loss of albumin-mediated control of intracellular calcium.[24] In addition, loss of syndecan expression hinders mechanotransduction signaling that is crucial to maintenance of cytoskeletal structure and junctional protein expression. Activated leukocytes secrete soluble factors that can directly disrupt paracellular vascular endothelial cadherin linkages. Furthermore, recent data from Richter et al.[25] demonstrate that heparan sulfate cleavage upregulates EC production of angiopoietin-2, resulting in suppression of Tie2 receptor–mediated maintenance of intercellular junction protein expression.

Contribution of Extracellular Vesicles to EoT

Endothelial cells subjected to traumatic, ischemic, and inflammatory insults undergo rapid changes that not only deplete the surface protective glycocalyx layer but also result in release of bioreactive molecules and EC apoptosis. These changes not only define trauma-induced endotheliopathy but also cause and disseminate secondary injuries following trauma. A key causal and disseminating factor is extracellular vesicle (EV) release from activated or apoptotic ECs that can be detected in peripheral blood samples of patients with trauma and in samples from experimental animals subjected to traumatic injury. These endothelial EVs have been widely used as markers for endothelial injury and, together with EVs from other cells, are increasingly recognized as a new class of biologic mediators.

Extracellular vesicles are ≤1 μm cellular vesicles and include membrane fragments, intracellular organelles, exosomes, and associated cargo molecules. They are released from cells undergoing apoptosis or active microvesiculation and can cause endothelial dysfunction and coagulopathy during acute injury through distinct but closely related pathways. First, membrane EVs often express anionic phospholipids, which are key cofactors in coagulation initiation and propagation. Second, EVs from peripheral blood samples of mice subjected to traumatic brain injury (TBI) induce vasoconstriction in vivo and in vitro, resulting in tissue ischemia.[26] This EV activity does not appear to derive from specific factors carried by the EVs but owing to the specific structure of EVs because neither the protein nor the lipid fraction extracted from these EVs induces vasoconstriction individually.[26] Third, EVs carry bioactive factors derived from parental cells or captured from plasma that can activate or injure ECs.

VWF in the Development of EoT

von Willebrand factor is synthesized primarily in ECs and megakaryocytes as a single-chain propolypeptide of 2,813 amino acids that contains multiple domains.[27] Pro-VWF first dimerizes through the C-terminal cystine knot (CK) domain. A variable number of dimers then multimerize through N-terminal disulfide bonds. Newly synthesized VWF multimers are either constitutively released into the circulation and subendothelial matrix or stored in the Weibel-Palade bodies of ECs and the ά-granules of megakaryocytes/platelets, where multimerization continues. As a result, VWF multimers stored in these granules are enriched in ultralarge forms and are released after ECs and platelets are activated.[28] von Willebrand factor multimers differ not only in molecular mass but also in adhesive activity.[27] von Willebrand factor circulating in the resting state binds platelets poorly unless they are immobilized on the exposed subendothelium at the site of vascular injury. In contrast, ultralarge von Willebrand factor (ULVWF) freshly released from the storage granules is intrinsically hyperadhesive and spontaneously binds platelets and ECs.[29] A key structural difference between the two forms of VWF is that the platelet-binding A1 domain is hidden in the globular structure of plasma VWF but exposed on the surface of hyperadhesive ULVWF.[30] Upon release, hyperadhesive ULVWF multimers are anchored to ECs, stretched by the shear stress of blood flow, and rapidly cleaved at the Y^{1605}-M^{1606} peptide bond in the A2 domain by the metalloprotease ADAMTS-13 (A Disintegrin And Metalloproteinase with a ThromboSpondin type 1 motif, member 13).[31] The cleavage releases smaller plasma forms of VWF multimers found in the circulation. The proteolysis therefore serves as a safeguard to maintain the hemostatic activity of plasma VWF without causing spontaneous thrombosis by ULVWF. This safeguard is lost or altered during acute trauma and hemorrhagic shock through multiple pathways. First, as an acute phase reactant, VWF is kept in the storage granules of ECs to ensure its quick release at the site of vascular injury. In contrast, ADAMTS-13 is constitutively released upon synthesis from hepatic stellate cells, ECs, and platelets.[32–34] As a result, the homeostatic balance between ADAMTS-13 and VWF is disrupted during acute trauma by the substantial release of VWF without a parallel release of ADAMTS-13. Second,

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ADAMTS-13 synthesis and activity are inhibited by inflammatory cytokines and post-translational citrullination.[35] Both VWF and ADAMTS-13 can also be oxidized in selective cysteine and methionine residues in the oxidative stress environment of trauma, rendering the former resistant to cleavage and the latter less able to cleave VWF.[36] Third, VWF cleavage by ADAMTS-13 is facilitated by the fluid shear stress of blood flow.[37] The cleavage is therefore likely suppressed in the hypoperfusion state caused by hemorrhagic shock. Finally, because of their multidomain multimeric structure, EV- and cell-bound VWF can serve as a coupling factor that tethers EVs to the surface of ECs through P-selectin, integrin $\alpha_v\beta_3$, or other molecules to enhance their vascular activities. Surface-anchored VWF multimers also bring leukocytes to ECs to facilitate or enhance vascular inflammation.

It is interesting that modifying VWF synthesis and release may affect endothelial integrity as VWF deficiency upregulated the tight junction protein claudin-5 to protect the integrity of the blood-brain barrier in mice.[38] Conversely, the microRNA miR-143-3p upregulated VWF expression to improve acute trauma-induced coagulopathy.[39] Desmopressin (DDAVP), which increases VWF release from the storage pool of ECs, along with a 1:1:1 transfusion strategy improved shock parameters and maintained coagulation in rats undergoing multiple injuries and shock compared with transfusion alone controls.[40] While the study attributed the protective effect of DDAVP to the release of VWF from ECs, it is equally possible that the drug depleted the intracellular pool of VWF, as found in VWF deficient ECs.[38] These observations thus raise the question whether intracellular (stored) and extracellular (released) VWF multimers differentially regulate endothelial integrity and function.

These research and clinical findings also raise the question of why and under what conditions the hemostatic factor VWF becomes detrimental in the acute trauma setting. Since plasma levels of VWF and ADAMTS-13 vary significantly among healthy individuals, one can speculate that subjects with intrinsically high VWF and/or low ADAMTS-13 are more likely to develop VWF-driven endotheliopathy and coagulopathy during acute trauma and hemorrhagic shock. For example, subjects with O blood type have low baseline levels of VWF, whereas blood type B subjects have the highest.[41] ABO blood group could therefore be a confounding factor for VWF-associated pathologies in acutely injured patients.[42] In a study of 268 patients, type O patients were more likely to develop hyperfibrinolysis than non-O patients and also had increased odds of massive transfusion after adjustment for injury severity score.[43] Preexisting conditions associated with hyperadhesive VWF could also exaggerate the detrimental activities of VWF. The role of coagulation factor VIII in trauma-induced endotheliopathy and coagulopathy is largely unknown, even though it circulates together as a complex with VWF and affects VWF cleavage. These knowledge gaps call for more extensive mechanistic studies to define the role of VWF in the pathogenesis of and recovery from trauma and hemorrhagic shock.

EoT Contributes to Traumatic Coagulopathy

As a key component of hemostasis, trauma-induced endothelial injury triggers local hemostasis at the site of injury. This process can become exaggerated and disseminated systemically, resulting in consumptive coagulopathy through several interconnected pathways. First, the endothelium, which provides an anticoagulant and platelet-repellent surface in the resting state becomes highly procoagulant and attracts platelets and leukocytes when its protective glycocalyx is depleted. Hyperadhesive VWF also tethers EVs and leukocytes to ECs remote from the injury site. Second, ECs express anionic phospholipids, such as phosphatidylserine on their surface and TF when they are activated or injured, to trigger coagulation on the surface of ECs, as demonstrated by fibrin deposition to the vessel wall.[44,45] As a transmembrane protein, endothelial EVs carry TF and phosphatidylserine to promote the formation of the tenase complex to generate thrombin, which is a potent EC activator, and to disseminate intravascular coagulation.[46] Although this is best described as occurring following TBI, increasing similarities between TBI and hemorrhagic shock-induced coagulation abnormalities are being identified. Third, VWF multimers, especially those with the exposed A1 domain, bind and activate platelets to promote microvascular thrombosis, intravascular coagulation, and the release of procoagulant and proinflammatory EVs. Fourth, the transmembrane TM (CD141) acts with protein C and EPCR to form the anticoagulant system on the surface of ECs. However, the shedding of TM from activated ECs by multiple enzymes weakens this anticoagulant system.[47] Soluble and EV-bound TM remains active, further increasing the risk of coagulopathy.[48] Similarly, shedding of heparan sulfates from the glycocalyx layer further diminishes EC anticoagulant functions, thereby upregulating thrombin production. Fifth, when perfused over cultured human ECs, plasma from trauma patients increased VWF release from ECs, and this activity was prevented by the antifibrinolytic agent tranexamic acid, providing a direct link between endotheliopathy and the state of fibrinolysis during acute trauma.[49] In addition, ADAMTS-13 activity measured in the early stage of trauma is significantly correlated with changes in prothrombin time and fibrin/fibrinogen degradation product, and ADAMTS-13 activity at <50% of normal levels correlates with the development of disseminated intravascular coagulation and the need for transfusion of fresh frozen plasma.[50] Finally, activated ECs release large intracellular pools of tPA, which has been shown to drive plasmin generation and hyperfibrinolysis in coagulopathic trauma patients. Collectively, these EC functional changes caused by EoT are likely to drive an early consumptive coagulopathic phenotype, driven by rapid upregulation of thrombin production and tPA-mediated fibrinolysis, which later shifts to a hypercoagulable state resulting from sustained EC activation in the postacute setting.[51]

POTENTIAL THERAPEUTICS TO MITIGATE THE EOT

Blood Products

Clinical studies demonstrating improved survival by the early use of plasma in patients in hemorrhagic shock led to the hypothesis that the benefit of plasma entailed more than just achieving hemostasis to also include protection of the endothelium.[52–54] In preclinical studies, plasma has been shown to restore the endothelial glycocalyx via restoration of syndecan-1.[17] Subsequent mechanistic studies sought to determine the molecule(s) in plasma responsible for its endothelial protective effects. Both AT III and adiponectin have been shown to contribute to plasma's

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protection of the endothelium.[55,56] Two separate groups of investigators determined that fibrinogen was also a key molecule.[57,58] Fibrinogen binds to cell surface endothelial syndecan-1 to stabilize it on the cell membrane, likely through binding to the heparan sulfate GAGs of the glycocalyx.[58] Wu et al.[59] demonstrated that this binding activates an intracellular PAK-1 signaling pathway, which disassembles stress fibers to reduce endothelial permeability and maintain barrier integrity. DeBot et al.[60] demonstrated that RhoA GTPase is activated after trauma and reduces endothelial permeability. RhoA GTPAse is upstream of PAK-1and likely is involved in the same pathway of endothelial protection. Because low fibrinogen levels are associated with worse outcomes after trauma, fibrinogen supplementation beyond that found in plasma has been investigated.[61–63] Correction of trauma-induced hypofibrinogenemia in the United States is primarily via cryoprecipitate administration; thus, studies in this country have focused on cryoprecipitate. An in vitro study by Wu et al.[64] found that cryoprecipitate restored EC barrier function comparable with fresh frozen plasma. This was followed by an in vivo study by Barry et al.[65] verifying cryoprecipitate's endothelial protection. The short postthaw storage time of cryoprecipitate, however, prevents its early use clinically. To address this limitation, there is now a pathogen-reduced 5-day postthaw shelf-life cryoprecipitate (intercept fibrinogen complex) and a lyophilized cryoprecipitate product that is under development. In a mouse model of multiple injuries and hemorrhagic shock, both conventional and lyophilized cryoprecipitate protected the endothelium comparable with plasma, while lyophilized cryoprecipitate further increased the ADAMTS13:VWF ratio beyond both conventional cryoprecipitate and plasma, suggesting potential additional benefit.[66] Both the 5-day shelf life and the lyophilized product also resulted in sustained organ protection in a long-term rodent model of hemorrhagic shock and prolonged hypotensive resuscitation.[67]

Clinical studies are ongoing to investigate the potential benefit of prothrombin complex concentrates on hemostasis after severe trauma. In a preclinical mouse model of hemorrhagic shock, Pati et al.[68] demonstrated that prothrombin complex concentrates comparably reduced vascular permeability with that of plasma.

Therapeutic Adjuncts to Blood Products

To date, efforts have focused on reversing endotheliopathy using blood component therapy as part of damage-control resuscitation, but additional adjunctive strategies are needed. Potential therapeutic adjuncts with implications for trauma because of their endothelial protective properties are presented.

Targeting the Glycocalyx

Given the important role of glycocalyx shedding in the pathogenesis of EoT, therapeutics that target responsible sheddases, or even upstream activation of leukocytes that produce such sheddases, have garnered increasing attention in a variety of preclinical models.[20] Examples of these include etanercept, angiopoetin-1, hydrocortisone, and heparin, although none of the reported benefits are specific to trauma.[20,69–71] In addition, in a clinical study, patients randomized to receiving doxycycline, an MMP inhibitor, exhibited reduced glycocalyx shedding in response to cardiopulmonary bypass.[72] While syndecan-1 provides structural support to the glycocalyx, the attached GAGs can also play an important role in protection. Heparan sulfate is the most abundant proteoglycan of the endothelial glycocalyx and is robustly shed in response to trauma and hemorrhagic shock. In particular, heparan sulfates endowed with a rare 3-O-sulfate modification possess both anticoagulant and anti-inflammatory properties through high affinity interaction with AT and the ability to bind and sequester circulation DAMPs. A recent preclinical study demonstrated that a synthetic 3-O sulfated heparan sulfate, dekaparin, had similar anti-inflammatory and organ protective properties to plasma in a mouse model of trauma and hemorrhagic shock.[73] These data indicate the important biological role of this unique heparan sulfate molecule and demonstrate that restoration of shed glycocalyx components could be a novel therapeutic avenue for future development.

Targeting ADAMTS-13 and VWF

von Willebrand factor–mediated EV-induced vascular activity after trauma can be blocked by exogenous ADAMTS-13, VWF antibody, or a recombinant A2 protein, which binds the A1 domain exposed on hyperadhesive VWF in rodent models of trauma and hemorrhagic shock or TBI.[36] These research findings are consistent with clinical observations that high plasma levels of VWF, low ADAMTS-13 activity, and/or an altered VWF-to-ADAMTS-13 ratios are associated with endotheliopathy, coagulopathy, and other measures linked to poor outcomes of patients with severe injury.[74–78] These findings suggest that repletion of ADAMTS-13 may be beneficial. A study by Zhou et al.[79] in a rodent model of renal ischemia/reperfusion found that recombinant human (rh) ADAMTS-13 reduced inflammation and improved EC function. Similarly, Wu et al.[80] found in a mouse model of TBI that rhADAMTS-13 was effective in mitigating cerebral vascular leakage and coagulopathy and improved neurologic outcomes and survival. Kleinveld et al.[74] examined rhADMATS13 in a rat model of shock and found that rhADAMTS13 decreased lung permeability and lung and kidney injury and minimized syndecan-1 shedding comparable with that of plasma. However, only rhADAMTS13 was able to restore the ADAMTS13:VWF ratio. There are several clinical trials ongoing or recently completed using rhADAMTS-13 in patients with thrombotic thrombocytopenic purpura and sickle cell, raising the possibility of repletion after trauma and hemorrhage may be feasible.

The platelet-binding A1 domain of VWF is exposed on hyperadhesive ULVWF multimers but is hidden in the globular structure of physiologic plasma VWF multimers. The A1 domain is hidden because it forms a complex with the adjacent A2 domain. This A1-A2 complex is formed when the two vicinal cysteine residues (C^{1669}-C^{1670}) in the A2 domain are reduced (free thiols), but it disassociates when these cysteines are oxidized.[81] A human recombinant A2 protein, which lacks the vicinal cysteines to allow binding of A1 in an oxidative environment such as that found in trauma, has been developed and is being tested. In models of sepsis, Nguyen et al.[82] found that rhA2 inhibited platelet interaction with fibrin to lessen intravascular thrombosis. To further explore its potential benefits, Xu et al.[83] administered rhA2 protein in a mouse model of TBI and found that it markedly reduced mortality and improved neurologic function. In addition, A2 protected the endothelium as

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evidenced by reduced vascular leakage in the brain, reduced lung edema, and reduced endothelial activation, which the authors attributed to a reduction of brain-derived EVs.[83]

Targeting EVs

von Willebrand factor multimers secreted after trauma and other pathologic conditions activate platelets and ECs to release procoagulant and proinflammatory EVs. These VWF-bound EVs provoke vascular hyperpermeability, a critical component of endotheliopathy. When EVs were isolated from severely injured patients and injected into naive mice, EC injury, as demonstrated by syndecan-1 shedding; coagulopathy; and organ injury were all significantly increased as compared with mice receiving EVs from minimally injured patients.[84] Similarly, Dyer et al.[85] found that platelet EVs released after trauma were prohemostatic but also prothrombotic and contributed to enhanced venous thrombosis.

Lactadherin (milk fat globule-epidermal growth factor 8) can remove pathologic EVs by coupling them to macrophages to facilitate phagocytosis. Lactadherin has an integrin-binding arginylglyclyaspartic acid sequence and two C-terminal domains that bind phosphatidyl serine with a high affinity. Zhou et al.[86] have shown that lactadherin promotes the clearance of EVs, lessens coagulopathy, prevents permeability, and improves neurologic outcomes and survival in mice subjected to severe TBI. Because trauma causes widespread tissue injury and global ischemia, it is likely that severely injured patients have more circulating EVs than those with isolated TBI. Lactadherin should therefore also prevent endotheliopathy and coagulopathy after trauma and hemorrhage, but this has not yet been studied.

Although EVs secreted after trauma are injurious, EVs can also be therapeutic when obtained under physiologic conditions.[87] For example, EVs from activated astrocytes carry miR-873a-5p, which inhibits nuclear factor κB signaling to reduce microglia-mediated neuroinflammation in TBI mice.[88] Extracellular vesicles from neutrophils increase the release of transforming growth factor β1 and downregulate macrophage activity to suppress injury-induced inflammation.[89] Platelet-derived EVs tempered development of hemorrhagic shock, stabilized the endothelium, and maintained EC barrier integrity.[90,91] An advantage of platelet EVs is that they can be stored frozen and potentially dried or lyophilized to extend their clinical applications.

Cellular Therapeutics

Mesenchymal stem cells (MSCs), which are stromal progenitor cells isolated from bone marrow, have been studied in vitro, in vivo, and clinically for use after trauma. They can facilitate angiogenesis to promote endothelial repair and, in models of hemorrhagic shock, can reduce endothelial permeability.[92] In addition, comparable protection was afforded by EV-derived MSCs as MSCs themselves, suggesting that MSC EVs may represent a viable cell-free therapeutic adjunct after trauma.[93,94] However, distinguishing the protective effects of EVs from their detrimental effects can be challenging because these diverse effects can be specific for parental cells, target cells, and cargo contents, that is, the same EVs can both injure and protect ECs. Continued efforts are therefore needed to minimize detrimental effects while enhancing the protective effects of EVs in regulating trauma pathogenesis.

In conclusion, the EoT involves a complex interplay between the glycocalyx, VWF, and platelets that lead to abnormalities in coagulation, inflammation, and EC function. The current review has focused on mechanisms of endothelial injury and the pathologic consequences of EoT as well as potential therapeutic targets. Most research to date has focused on blood products, primarily plasma, to restore EC dysfunction, although a number of additional adjuncts are now being investigated. Much of the pathobiology of EoT is known, but a better mechanistic understanding can help guide therapeutics to further repair the EoT and improve patient outcomes.

AUTHORSHIP

J.C.C., J.F.D., and R.A.K. contributed in the content, writing, and editing of the manuscript.

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DISCLOSURE

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REFERENCES

1. Mast AE. Tissue factor pathway inhibitor: multiple anticoagulant activities for a single protein. *Arterioscler Thromb Vasc Biol*. 2016;36(1):9–14.
2. Jackson SP, Darbousset R, Schoenwaelder SM. Thromboinflammation: challenges of therapeutically targeting coagulation and other host defense mechanisms. *Blood*. 2019;133(9):906–918.
3. Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. *Nat Rev Immunol*. 2007;7(10):803–815.
4. Gimbrone MA Jr., García-Cardeña G. Endothelial cell dysfunction and the pathobiology of atherosclerosis. *Circ Res*. 2016;118(4):620–636.
5. Pretorius D, Richter RP, Anand T, Cardenas JC, Richter JR. Alterations in heparan sulfate proteoglycan synthesis and sulfation and the impact on vascular endothelial function. *Matrix Biol Plus*. 2022;16:100121.
6. Wang G, Tiemeier GL, van den Berg BM, Rabelink TJ. Endothelial glycocalyx hyaluronan: regulation and role in prevention of diabetic complications. *Am J Pathol*. 2020;190(4):781–790.
7. Hayashida K, Stahl PD, Park PW. Syndecan-1 ectodomain shedding is regulated by the small GTPase Rab5. *J Biol Chem*. 2008;283(51):35435–35444.
8. Jenkins DH, Rappold JF, Badloe JF, Berséus O, Blackbourne L, Brohi KH, et al. Trauma hemostasis and oxygenation research position paper on remote damage control resuscitation: definitions, current practice, and knowledge gaps. *Shock*. 2014;41(Suppl 1(0 1)):3–12.
9. Johansson PI, Henriksen HH, Stensballe J, Gybel-Brask M, Cardenas JC, Baer LA, et al. Traumatic endotheliopathy: a prospective observational study of 424 severely injured patients. *Ann Surg*. 2017;265(3):597–603.
10. Haywood-Watson RJ, Holcomb JB, Gonzalez EA, Peng Z, Pati S, Park PW, et al. Modulation of syndecan-1 shedding after hemorrhagic shock and resuscitation. *PloS One*. 2011;6(8):e23530.
11. Johansson PI, Stensballe J, Rasmussen LS, Ostrowski SR. A high admission syndecan-1 level, a marker of endothelial glycocalyx degradation, is associated with inflammation, protein C depletion, fibrinolysis, and increased mortality in trauma patients. *Ann Surg*. 2011;254(2):194–200.
12. Gonzalez Rodriguez E, Ostrowski SR, Cardenas JC, Baer LA, Tomasek JS, Henriksen HH, et al. Syndecan-1: a quantitative marker for the endotheliopathy of trauma. *J Am Coll Surg*. 2017;225(3):419–427.
13. Naumann DN, Hazeldine J, Davies DJ, Bishop J, Midwinter MJ, Belli A, et al. Endotheliopathy of trauma is an on-scene phenomenon and is associated with multiple organ dysfunction syndrome: a prospective observational study. *Shock*. 2018;49(4):420–428.
14. Zeineddin A, Wu F, Chao W, Zou L, Vesselinov R, Chipman AM, et al. Biomarkers of endothelial cell dysfunction persist beyond resuscitation in patients with hemorrhagic shock. *J Trauma Acute Care Surg*. 2022;93(5):572–578.

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15. Krocker JD, Lee KH, Henriksen HH, Wang YW, Schoof EM, Karvelsson ST, et al. Exploratory investigation of the plasma proteome associated with the endotheliopathy of trauma. *Int J Mol Sci*. 2022;23(11):6213.
16. Rahbar E, Cardenas JC, Baimukanova G, Usadi B, Bruhn R, Pati S, et al. Endothelial glycocalyx shedding and vascular permeability in severely injured trauma patients. *J Transl Med*. 2015;13:117.
17. Kozar RA, Peng Z, Zhang R, Holcomb JB, Pati S, Park P, et al. Plasma restoration of endothelial glycocalyx in a rodent model of hemorrhagic shock. *Anesth Analg*. 2011;112(6):1289–1295.
18. Torres Filho I, Torres LN, Sondeen JL, Polykratis IA, Dubick MA. In vivo evaluation of venular glycocalyx during hemorrhagic shock in rats using intravital microscopy. *Microvasc Res*. 2013;85:128–133.
19. Ramnath R, Foster RR, Qiu Y, Cope G, Butler MJ, Salmon AH, et al. Matrix metalloproteinase 9–mediated shedding of syndecan 4 in response to tumor necrosis factor α: a contributor to endothelial cell glycocalyx dysfunction. *FASEB J*. 2014;28(11):4686–4699.
20. Becker BF, Jacob M, Leipert S, Salmon AHJ, Chappell D. Degradation of the endothelial glycocalyx in clinical settings: searching for the sheddases. *Br J Clin Pharmacol*. 2015;80(3):389–402.
21. Masola V, Greco N, Gambaro G, Franchi M, Onisto M. Heparanase as active player in endothelial glycocalyx remodeling. *Matrix Biol Plus*. 2021;13:100097.
22. Goodall KJ, Poon IKH, Phipps S, Hulett MD. Soluble heparan sulfate fragments generated by heparanase trigger the release of pro-inflammatory cytokines through TLR-4. *PloS One*. 2014;9(10):e109596.
23. Dogné S, Flamion B. Endothelial glycocalyx impairment in disease: focus on Hyaluronan shedding. *Am J Pathol*. 2020;190(4):768–780.
24. Dull RO, Hahn RG. The glycocalyx as a permeability barrier: basic science and clinical evidence. *Crit Care*. 2022;26(1):273.
25. Richter RP, Ashtekar AR, Zheng L, Pretorius D, Kaushlendra T, Sanderson RD, et al. Glycocalyx heparan sulfate cleavage promotes endothelial cell angiopoietin-2 expression by impairing shear stress-related AMPK/FoxO1 signaling. *JCI Insight*. 2022;7(15):e155010.
26. Wang J, Xie X, Wu Y, Zhou Y, Li Q, Li Y, et al. Brain-derived extracellular vesicles induce vasoconstriction and reduce cerebral blood flow in mice. *J Neurotrauma*. 2022;39(11–12):879–890.
27. Sadler JE. Biochemistry and genetics of von Willebrand factor. *AnnuRevBiochem*. 1998;67:395–424.
28. Moake JL, Chow TW. Increased von Willebrand factor (vWf) binding to platelets associated with impaired vWf breakdown in thrombotic thrombocytopenic purpura. *J Clin Apher*. 1998;13(3):126–132.
29. Furlan M. Von Willebrand factor: molecular size and functional activity. *Ann Hematol*. 1996;72(6):341–348.
30. Singh I, Shankaran H, Beauharnois ME, Xiao Z, Alexandridis P, Neelamegham S. Solution structure of human von Willebrand factor studied using small angle neutron scattering. *J Biol Chem*. 2006;281(50):38266–38275.
31. Levy GG, Nichols WC, Lian EC, Foroud T, McClintick JN, McGee BM, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature*. 2001;413(6855):488–494.
32. Uemura M, Tatsumi K, Matsumoto M, Fujimoto M, Matsuyama T, Ishikawa M, et al. Localization of ADAMTS13 to the stellate cells of human liver. *Blood*. 2005;106(3):922–924.
33. Turner N, Nolasco L, Tao Z, Dong JF, Moake J. Human endothelial cells synthesize and release ADAMTS-13. *J Thromb Haemost*. 2006;4(6):1396–1404.
34. Liu L, Choi H, Bernardo A, Nolasco C, Ruan C, Moake JL, et al. Platelet-derived VWF-cleaving metalloprotease ADAMTS-13. *J Thromb Haemost*. 2005;3(11):2536–2544.
35. Sorvillo N, Mizurini DM, Coxon C, Martinod K, Tilvawala R, Cherpokova D, et al. Plasma peptidylarginine deiminase IV promotes VWF-platelet string formation and accelerates thrombosis after vessel injury. *Circ Res*. 2019;125(5):507–519.
36. Xu X, Kozar R, Zhang J, Dong JF. Diverse activities of von Willebrand factor in traumatic brain injury and associated coagulopathy. *J Thromb Haemost*. 2020;18(12):3154–3162.
37. Wu T, Lin J, Cruz MA, Dong JF, Zhu C. Force-induced cleavage of single VWFA1A2A3 tridomains by ADAMTS-13. *Blood*. 2010;115(2):370–378.
38. Suidan GL, Brill A, De Meyer SF, Voorhees JR, Cifuni SM, Cabral JE, et al. Endothelial Von Willebrand factor promotes blood-brain barrier flexibility and provides protection from hypoxia and seizures in mice. *Arterioscler Thromb Vasc Biol*. 2013;33(9):2112–2120.
39. Luo Y, Liu S, Li Q, Zhang Y, Fu Y, Yang N, et al. circ_0001274 Competitively binds miR-143-3p to upregulate VWF expression to improve acute traumatic coagulopathy. *Oxid Med Cell Longev*. 2023;2023:9650323.
40. Wirtz MR, Roelofs JJ, Goslings JC, Juffermans NP. Treatment with ddAVP improves platelet-based coagulation in a rat model of traumatic hemorrhagic shock. *Trauma Surg Acute Care Open*. 2022;7(1):e000852.
41. Song J, Chen F, Campos M, Bolgiano D, Houck K, Chambless LE, et al. Quantitative influence of ABO blood groups on factor VIII and its ratio to von Willebrand factor, novel observations from an ARIC study of 11,673 subjects. *PloS One*. 2015;10(8):e0132626.
42. Muellner SK, Haut ER, Streiff MB, Holcomb JB, Cotton BA. ABO blood group as a potential risk factor for venous thromboembolism in acutely injured patients. *Thromb Haemost*. 2011;105(1):5–13.
43. DeBot M, Eitel AP, Moore EE, Sauaia A, Lutz P, Schaid TR, et al. Blood type O is a risk factor for hyperfibrinolysis and massive transfusion after severe injury. *Shock*. 2022;58(6):492–497.
44. Stojkovic S, Kaun C, Basilio J, Rauscher S, Hell L, Krychtiuk KA, et al. Tissue factor is induced by interleukin-33 in human endothelial cells: a new link between coagulation and inflammation. *Sci Rep*. 2016;6:25171.
45. Tian Y, Salsbery B, Wang M, Yuan H, Yang J, Zhao Z, et al. Brain-derived microparticles induce systemic coagulation in a murine model of traumatic brain injury. *Blood*. 2015;125(13):2151–2159.
46. Dong X, Liu W, Shen Y, Houck K, Yang M, Zhou Y, et al. Anticoagulation targeting membrane-bound anionic phospholipids improves outcomes of traumatic brain injury in mice. *Blood*. 2021;138(25):2714–2726.
47. Boron M, Hauzer-Martin T, Keil J, Sun XL. Circulating thrombomodulin: release mechanisms, measurements, and levels in diseases and medical procedures. *TH Open*. 2022;6(3):e194–e212.
48. Andriantsitohaina R, Gaceb A, Vergori L, Martinez MC. Microparticles as regulators of cardiovascular inflammation. *Trends Cardiovasc Med*. 2012;22(4):88–92.
49. Diebel LN, Liberati DM. Effect of tranexamic acid on endothelial von Willebrand factor/ADAMTS-13 response to in vitro shock conditions. *J Trauma Acute Care Surg*. 2023;94(2):273–280.
50. Matsumoto H, Takeba J, Umakoshi K, Kikuchi S, Ohshita M, Annen S, et al. ADAMTS13 activity decreases in the early phase of trauma associated with coagulopathy and systemic inflammation: a prospective observational study. *Thromb J*. 2021;19(1):17.
51. Cardenas JC, Matijevic N, Baer LA, Holcomb JB, Cotton BA, Wade CE. Elevated tissue plasminogen activator and reduced plasminogen activator inhibitor promote hyperfibrinolysis in trauma patients. *Shock*. 2014;41(6):514–521.
52. Holcomb JB, del Junco DJ, Fox EE, Wade CE, Cohen MJ, Schreiber MA, Alarcon LH, Bai Y, Brasel KJ, Bulger EM, et al, and PROMMTT Study Group. The prospective, observational, multicenter, major trauma transfusion (PROMMTT) study: comparative effectiveness of a time-varying treatment with competing risks. *JAMA Surg*. 2013;148(2):127–136.
53. Holcomb JB, Tilley BC, Baraniuk S, Fox EE, Wade CE, Podbielski JM, del Junco DJ, Brasel KJ, Bulger EM, Callcut RA, et al, and the PROPPR Study Group. Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma: the PROPPR randomized clinical trial. *JAMA*. 2015;313(5):471–482.
54. Sperry JL, Guyette FX, Brown JB, Yazer MH, Triulzi DJ, Early-Young BJ, Adams PW, Daley BJ, Miller RS, Harbrecht BG, et al, PAMPer Study Group. Prehospital plasma during air medical transport in trauma patients at risk for hemorrhagic shock. *N Engl J Med*. 2018;379(4):315–326.
55. Lopez E, Peng Z, Kozar RA, Cao Y, Ko TC, Wade CE, et al. Antithrombin III contributes to the protective effects of fresh frozen plasma following hemorrhagic shock by preventing Syndecan-1 shedding and endothelial barrier disruption. *Shock*. 2020;53(2):156–163.
56. Deng X, Cao Y, Huby MP, Duan C, Baer L, Peng Z, et al. Adiponectin in fresh frozen plasma contributes to restoration of vascular barrier function after hemorrhagic shock. *Shock*. 2016;45(1):50–54.
57. Yu Q, Yang B, Davis JM, Ghosn J, Deng X, Doursout MF, et al. Identification of fibrinogen as a key anti-apoptotic factor in human fresh frozen plasma for protecting endothelial cells in vitro. *Shock*. 2020;53(5):646–652.
58. Wu F, Kozar RA. Fibrinogen protects against barrier dysfunction through maintaining cell surface syndecan-1 in vitro. *Shock*. 2019;51(6):740–744.
59. Wu F, Chipman A, Dong JF, Kozar RA. Fibrinogen activates PAK1/Cofilin signaling pathway to protect endothelial barrier integrity. *Shock*. 2021;55(5):660–665.
60. DeBot M, Mitra S, Lutz P, Schaid TR Jr., Stafford P, Hadley JB, et al. Shock induces endothelial permeability after trauma through increased activation of RHOA GTPASE. *Shock*. 2022;58(6):542–548.
61. Richards J, Fedeles BT, Chow JH, Scalea T, Kozar R. Raising the bar on fibrinogen: a retrospective assessment of critical hypofibrinogenemia in

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severely injured trauma patients. *Trauma Surg Acute Care Open*. 2023; 8(1):e000937.
62. Inaba K, Karamanos E, Lustenberger T, Schöchl H, Shulman I, Nelson J. Impact of fibrinogen levels on outcomes after acute injury in patients requiring a massive transfusion. *J Am Coll Surg*. 2013;216(2):290–297.
63. McQuilten ZK, Wood EM, Bailey M, Cameron PA, Cooper DJ. Fibrinogen is an independent predictor of mortality in major trauma patients: a five-year statewide cohort study. *Injury*. 2017;48(5):1074–1081.
64. Wu F, Chipman A, Pati S, Miyasawa B, Corash L, Kozar RA. Resuscitative strategies to modulate the endotheliopathy of trauma: from cell to patient. *Shock*. 2020;53(5):575–584.
65. Barry M, Trivedi A, Miyazawa BY, Vivona LR, Khakoo M, Zhang H, et al. Cryoprecipitate attenuates the endotheliopathy of trauma in mice subjected to hemorrhagic shock and trauma. *J Trauma Acute Care Surg*. 2021;90(6): 1022–1031.
66. Zeineddin A, Wu F, Dong J-F, Vesselinov R, Neal MD, Corash L, et al. Early lyophilized cryoprecipitate enhances the ADAMTS13: VWF ratio to reduce systemic endotheliopathy and lessen lung injury in a mouse polytrauma hemorrhage model: lyophilized cryoprecipitate protects after hemorrhage [published online May 22, 2023]. *J Trauma Acute Care Surg*. doi:10.1097/TA.0000000000004065.
67. Zeineddin A, Wu F, Cao S, Corash L, Pati S, Kozar RA. Immediate use cryoprecipitate products provide lasting organ protection in a rodent model of trauma/hemorrhagic shock and prolonged hypotensive resuscitation [published online June 14, 2023]. *J Trauma Acute Care Surg*. doi:10.1097/TA.0000000000003981.
68. Pati S, Potter DR, Baimukanova G, Farrel DH, Holcomb JB, Schreiber MA. Modulating the endotheliopathy of trauma: factor concentrate versus fresh frozen plasma. *J Trauma Acute Care Surg*. 2016;80(4):576–584; discussion 584-5.
69. Salmon AHJ, Neal CR, Sage LM, Glass CA, Harper SJ, Bates DO. Angiopoietin-1 alters microvascular permeability coefficients in vivo via modification of endothelial glycocalyx. *Cardiovasc Res*. 2009;83(1):24–33.
70. Chappell D, Dörfler N, Jacob M, Rehm M, Welsch U, Conzen P, et al. Glycocalyx protection reduces leukocyte adhesion after ischemia/reperfusion. *Shock*. 2010;34(2):133–139.
71. Lipowsky HH, Lescanic A. Inhibition of inflammation induced shedding of the endothelial glycocalyx with low molecular weight heparin. *Microvasc Res*. 2017 Jul;112:72–78.
72. Gao W, Fang F, Xia TJ, Zhang Y, Sun J, Wu Q, et al. Doxycycline can reduce glycocalyx shedding by inhibiting matrix metalloproteinases in patients undergoing cardiopulmonary bypass: a randomized controlled trial. *Microvasc Res*. 2022;142:104381.
73. Vidaurre MDPH, Osborn BK, Lowak KD, McDonald MM, Wang YWW, Pa V, et al. A 3-*O*-sulfated heparan sulfate dodecasaccharide (12-mer) suppresses thromboinflammation and attenuates early organ injury following trauma and hemorrhagic shock. *Front Immunol*. 2023;14:1158457.
74. Kleinveld DJB, Simons DDG, Dekimpe C, Deconinck SJ, Sloos PH, Maas MAW, Kers J, Muia J, Brohi K, Voorberg J, et al. BloodNet. Plasma and rhADAMTS13 reduce trauma-induced organ failure by restoring the ADAMTS13-VWF axis. *Blood Adv*. 2021;5(17):3478–3491.
75. Taylor A, Vendramin C, Singh D, Brown MM, Scully M. von Willebrand factor/ADAMTS13 ratio at presentation of acute ischemic brain injury is predictive of outcome. *Blood Adv*. 2020;4(2):398–407.
76. Plautz WE, Raval JS, Dyer MR, Rollins-Raval MA, Zuckerbraun BS, Neal MD. ADAMTS13: origins, applications, and prospects. *Transfusion*. 2018; 58(10):2453–2462.
77. Dyer MR, Plautz WE, Ragni MV, Alexander W, Haldeman S, Sperry JL, Guyette FX, Zuckerbraun BS, Rollins-Raval MA, Raval JS, et al, A TACTIC Publication. Traumatic injury results in prolonged circulation of ultralarge von Willebrand factor and a reduction in ADAMTS13 activity. *Transfusion*. 2020;60(6):1308–1318.
78. Russell RT, McDaniel JK, Cao W, Shroyer M, Wagener BM, Zheng XL, et al. Low plasma ADAMTS13 activity is associated with coagulopathy, endothelial cell damage and mortality after severe paediatric trauma. *Thromb Haemost*. 2018;118(4):676–687.
79. Zhou S, Jiang S, Guo J, Xu N, Wang Q, Zhang G, et al. ADAMTS13 protects mice against renal ischemia-reperfusion injury by reducing inflammation and improving endothelial function. *Am J Physiol Renal Physiol*. 2019; 316(1):F134–F145.
80. Wu Y, Liu W, Zhou Y, Hilton T, Zhao Z, Liu W, et al. von Willebrand factor enhances microvesicle-induced vascular leakage and coagulopathy in mice with traumatic brain injury. *Blood*. 2018;132(10):1075–1084.
81. Martin C, Morales LD, Cruz MA. Purified A2 domain of von Willebrand factor binds to the active conformation of von Willebrand factor and blocks the interaction with platelet glycoprotein Ibalpha. *J Thromb Haemost*. 2007; 5(7):1363–1370.
82. Nguyen TC, Gushiken F, Correa JI, Dong JF, Dasgupta SK, Thiagarajan P, et al. A recombinant fragment of von Willebrand factor reduces fibrin-rich microthrombi formation in mice with endotoxemia. *Thromb Res*. 2015; 135(5):1025–1030.
83. Xu X, Wang C, Wu Y, Houck K, Hilton T, Zhou A, et al. Conformation-dependent blockage of activated VWF improves outcomes of traumatic brain injury in mice. *Blood*. 2021;137(4):544–555.
84. Zeineddin A, Wu F, Dong JF, Huang H, Zou L, Chao W, et al. Trauma-derived extracellular vesicles are sufficient to induce endothelial dysfunction and coagulopathy. *Shock*. 2022;58(1):38–44.
85. Dyer MR, Alexander W, Hassoune A, Chen Q, Brzoska T, Alvikas J, et al. Platelet-derived extracellular vesicles released after trauma promote hemostasis and contribute to DVT in mice. *J Thromb Haemost*. 2019;17(10):1733–1745.
86. Zhou Y, Cai W, Zhao Z, Hilton T, Wang M, Yeon J, et al. Lactadherin promotes microvesicle clearance to prevent coagulopathy and improves survival of severe TBI mice. *Blood*. 2018;131(5):563–572.
87. Alsaadi N, Srinivasan AJ, Seshadri A, Shiel M, Neal MD, Scott MJ. The emerging therapeutic potential of extracellular vesicles in trauma. *J Leukoc Biol*. 2022;111(1):93–111.
88. Long X, Yao X, Jiang Q, Yang Y, He X, Tian W, et al. Astrocyte-derived exosomes enriched with miR-873a-5p inhibit neuroinflammation via microglia phenotype modulation after traumatic brain injury. *J Neuroinflammation*. 2020;17(1):89.
89. Gasser O, Schifferli JA. Activated polymorphonuclear neutrophils disseminate anti-inflammatory microparticles by ectocytosis. *Blood*. 2004;104(8):2543–2548.
90. Lopez E, Srivastava AK, Burchfield J, Wang YW, Cardenas JC, Togarrati PP, et al. Platelet-derived extracellular vesicles promote hemostasis and prevent the development of hemorrhagic shock. *Sci Rep*. 2019;9(1):17676.
91. Miyazawa B, Trivedi A, Togarrati PP, Potter D, Baimukanova G, Vivona L, et al. Regulation of endothelial cell permeability by platelet-derived extracellular vesicles. *J Trauma Acute Care Surg*. 2019;86(6):931–942.
92. Matthay MA, Pati S, Lee JW. Concise review: mesenchymal stem (stromal) cells: biology and preclinical evidence for therapeutic potential for organ dysfunction following trauma or sepsis. *Stem Cells*. 2017;35(2):316–324.
93. Potter DR, Miyazawa BY, Gibb SL, Deng X, Togaratti PP, Croze RH, et al. Mesenchymal stem cell–derived extracellular vesicles attenuate pulmonary vascular permeability and lung injury induced by hemorrhagic shock and trauma. *J Trauma Acute Care Surg*. 2018;84(2):245–256.
94. Barry M, Trivedi A, Pathipati P, Miyazawa BY, Vivona LR, Togarrati PP, et al. Mesenchymal stem cell extracellular vesicles mitigate vascular permeability and injury in the small intestine and lung in a mouse model of hemorrhagic shock and trauma. *J Trauma Acute Care Surg*. 2022;92(3):489–498.

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